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A Compound Containing a Labile Disulfide Bond**CROSS-REFERENCE TO RELATED APPLICATIONS**

- 5 [(Provisional Application Serial No.) (Filing Date)]
This application claims priority benefit of U.S. Provisional Applications Serial No.
60/085,764 filed May 16, 1998 [5/16/98].

FEDERALLY SPONSORED RESEARCH

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N/A

Background

- 15 Bifunctional molecules, commonly referred to as crosslinkers, are used to connect two molecules together. Bifunctional molecules can contain homo or heterobifunctionality. The disulfide linkage (RSSR') may be used within bifunctional molecules. The reversibility of disulfide bond formation makes them useful tools for the transient attachment of two molecules. Disulfides have been used to attach a
- 20 bioactive compound and another compound (Thorpe, P.E. *J. Natl. Cancer Inst.* **1987**, *79*, 1101). The disulfide bond is reduced thereby releasing the bioactive compound. Disulfide bonds may also be used in the formation of polymers (Kishore, K., Ganesh, K. in *Advances in Polymer Science*, Vol. 21, Saegusa, T. Ed., 1993).
- 25 There are many commercially available reagents for the linkage of two molecules by a disulfide bond. Additionally there are bifunctional reagents that have a disulfide bond present. Typically, these reagents are based on 3-mercaptopropionic acid, i.e. dithiobispropionate. However, the rate at which these bonds are broken under physiological conditions is slow. For example, the half life of a disulfide derived
- 30 from dithiobispropionimide, an analog of 3-mercaptopropionic acid, is 27 hours in vivo (Arpicco, S., Dosio, F., Brusa, P., Crosasso, P., Cattel, L. *Bioconjugate Chem.* **1997**, *8*, 327.). A stable disulfide bond is often desirable, for example when purification of linked molecules or long circulation *in vivo* is needed. For this reason, attempts have been made to make the disulfide less susceptible to cleavage.

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1,4-bis(3-aminopropyl)piperazine (5.0 μ L, 0.023 mmol, Aldrich Chemical Company) and folate monomer (5.0 mg, 0.0012 mmol) were taken up in 0.4 mL methanol and HCl (1 mL, 1 M in Et₂O, Aldrich Chemical Company) was added. The resulting suspension was concentrated under reduced pressure to afford a white solid.

- 5 The salt was taken up in 0.5 mL DMF and 5,5'-dithiobis[succinimidyl(2-nitrobenzoate)] (14 mg, 0.025 mmol) was added. The resulting solution was heated to 80 °C and diisopropylethylamine (18 μ L, 0.10 mmol, Aldrich Chemical Company) was added by drops. After 16 hr, the solution was cooled, diluted with 3 mL H₂O, and dialyzed in 12,000 – 14,000 MW cutoff tubing against water (2 X 2 L) for 24 h.
- 10 The solution was then removed from dialysis tubing and dried by lyophilization to yield 13 mg (68%) of 5,5'-dithiobis(2-nitrobenzoic acid) - 1,4-bis(3-aminopropyl) piperazine -- folate copolymer.

- 15 Example 35: Synthesis of 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer

- | H₂N-EEEEEEEE-NHCH₂CH₂NH₂ (SEQ ID #1; 5.0 mg, 0.0052 mmol, Genosis) was taken up in 0.1 mL HEPES (250 mM, pH 7.5). 5,5'-dithiobis[succinimidyl(2-nitrobenzoate)] (3.1 mg, 0.0052) was added with 0.2 mL DMSO and the mixture was
- 20 stirred overnight at room temperature. After 16 hr the solution was heated to 70°C for 10 min, cooled to room temperature and diluted to 1.10 mL with DMSO.

- Example 36: Complex Formation with 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer
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- Fluorescein labeled DNA was used for the determination of DNA condensation in complexes with 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer. pDNA was modified to a level of 1 fluorescein per 20 bases using Mirus'
- 30 *LabelIT*TM Fluorescein kit. The fluorescence was determined using a fluorescence spectrophotometer (Shimadzo RF-1501 Fluorescence Spectrophotometer), at an excitation wavelength of 497 nm, and an emission wavelength of 520 nm.

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